"RELATIONSHIP BETWEEN CARIES STATUS (CARIES AND CARIES FREE GROUP), SALIVARY FLOW RATE, BUFFERING CAPACITY, SALIVARY MUTANS STREPTOCOCCUS COUNT AND SUGAR INTAKE AMONG 3-5 YEAR OLD PRESCHOOL CHILDREN"

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In partial fulfillment for the Degree of

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BRANCH VIII

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CERTIFICATE

This is to certify that this dissertation titled **"Relationship between caries status (caries and caries free group), salivary flow rate, buffering capacity, salivary mutans streptococcus count and sugar intake among 3-5 year old preschool children"** is a bonafide record of work done by **Dr. N. Uma Maheswari** under my guidance during her postgraduate study period between 2004 – 2007.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in Partial fulfillment for the Degree of **Master of Dental Surgery in Branch VIII – Pedodontics & Preventive Dentistry.**

It has not been submitted (partial or full) for the award of any other degree or diploma.

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By

Dr. N. UMA MAHESWARI

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Introduction

INTRODUCTION

In a period marked by brilliant achievements in the prevention and treatment of disease, dental caries still remains one of the most widespread affliction in modern man[35]. Dental caries is a slowly progressing disease that manifests as a result of mineral imbalance between the tooth and the environment. It is something of a paradox that teeth can be destroyed relatively rapidly in vivo and are almost indestructible postmortem. While it is true that diseases of teeth do not normally kill humans, they certainly affect the person's efficiency and they can, if neglected, provoke serious conditions elsewhere in the body. Their contribution to the general fund of human misery is legendary.

The disease progression can be stopped if the factors responsible are nullified in the initial stage. It is thus important to identify high caries risk markers as well as individuals to implement preventive and interceptive procedures.

Dental caries is a multifactorial disease in which there is interplay of three principle factors; the host (primarily the saliva and teeth), the microflora, and the substrate or diet. In evaluating the caries risk of a patient a number of factors must be taken into consideration. Salivary counts of mutants streptococcus combined with the measurement of salivary flow rate and buffer effect and sugar consumption are frequently used for diagnostic and predictive purpose in cariology[21].

Saliva is one of our natural resource. “For many years now it has been recognized that the quantity of saliva has some effect upon the teeth. As to that exactly the effect is, however, there is considerable divergence of opinion”. – This statement was made by ***Pickerill*** [34] in Otago,New Zealand in the year 1912 ***Miller*** [32] recorded that the flow of saliva plays an important role in the pathogenesis of dental caries.He proved the fact that the individuals with the diminished flow of saliva developed severe, rapid spreading caries lesion. Since then, numerous investigators have demonstrated that severe impairment of salivary secretion results in marked increase in the incidence of caries.

The relationship of flow rate to caries is further compounded where one considers the matter of buffering capacity of saliva. The salivary buffer capacity is an important host factor to maintain a suitable pH with its bicarbonate content in saliva. Its concentration in the saliva is positively correlated to the salivary flow rate, and a low or extremely low buffer capacity has been reported to correlate with the increased dental caries experience[12].

The micro flora involved in the dental caries activity is streptococcus mutans. It has a unique feature of producing extra cellular polysaccharides eg.dextran or glucan which increases the tenacity of plaque as well as limits the diffusion of acids which increases the acidity to bring about demineralization of tooth enamel[54].

Alaluusua and Rennonen (1983)[2] demonstrated that the early establishment of s.mutans indicates a high caries risk. These investigators found that children who harbor s.mutans in their plaque at the age of 2 years are likely to be more caries active and have significantly higher dmfs at 4 years than children who harbor s.mutans later ,or remain free from s.mutans infection. They concluded that early establishment of s.mutans in the plaque of primary incisors indicates potentially early and extensive caries attack in the primary dentition.

Similar studies done on salivary s.mutants by ***Catalanotto et al 1975*** [8]**,** ***Kocher et al 1984*** [6]**,** ***Newbrun et al 1984*** [33] demonstrated that children with high mutans counts had high dmfs increments over the period of 4 years. Thus there appears to be increasing evidence that s.mutans may be important in predicting high caries level in the children.

It has been accepted for many years, and almost without question since the Vipeholm study, that sugar consumption is a major risk factor for dental caries[46]. The presence of sucrose or other fermentable carbohydrates favour colonization of the dentition by acidogenic microorganisms. In developing countries an increase in the consumption of sugary foods or changes in eating habits have been associated with the dental caries[31].

Based on the above facts, it clearly indicates that, the predictive value of a single diagnostic test on an individual can be misleading in disease of a multifactorial nature such as dental caries[7]. Hence this study was undertaken to

seek the relationship of salivary microbiologic parameters.(mutans streptococcus count) and salivary physiochemical parameters (flow rate, buffering capacity) and sucrose intake with the occurrence of dental decay on the primary dentition among 3-5 years old of preschool children. These parameters were taken as assays that could be used as risk indicators of dental caries.The 3-5 yrs age groups presented with their primary dentition could be of interest because controlling the level of s.mutans in the early age could be beneficial, so that the upcoming dentition would not be challenged by cariogenic ability of these organisms.

Aims and Objectives

AIMS AND OBJECTIVES

1. To study the relation between caries status, salivary flow rate, salivary buffering capacity, mutans streptococcus count, and sugar intake among 3-5 year old pre school children.
2. To correlate the frequency of sugar intake with caries experience of the individual.
3. To determine the relation between salivary flow rate and salivary buffering capacity with caries experience.
4. To compare the levels of s.mutans with the caries experience of the individual.
5. To evaluate the relationship, if any between microbial parameter and sugar index.
6. To evaluate the relationship, if any between microbial parameter and salivary parameters.
7. To identify the caries risk group and to implement preventive strategies on the primary dentition so as to prevent the transmission of an infection from the primary to permanent dentition, subsequently interfering with or delaying the occurrence of decay.

Review of Literature

REVIEW OF LITERATURE

In 1924 Clarke isolated the streptococcus that predominated in many human carious lesions and that he named as “Streptococcus mutans” because of its varying morphology. Clarke noted that S.mutans adhered closely to tooth surfaces in artificially induced caries. For the next 40 years, S.mutans was virtually ignored, until the 1960s when it was “rediscovered” and its prevalence in plaque confirmed. The property of forming insoluble extracellular polysaccharides from sucrose is regarded as an important characteristic contributing to the caries-inducing properties of S.mutans. The use of salivary mutans streptococci (SMS) counts as a predictor of dental caries in the primary dentition has received much attention over the past decade. Several studies have shown a direct relationship between dental caries and SMS level [Chosack et al., 1988; Edelstein and Tinanoff, 1989; Holbrook et al., 1989; Bretz et al., 1992; Thibodeau et al., 1993].

Culturing plaque samples from discrete sites, such as occlusal fissures or proximal area, is an ideal method for the purpose of detecting and quantitating the mutans streptococcus that have colonised on teeth. However, it is often not practical to sample a large number of dental sites, hence the use of salivary sample to provide a workable alternative to assaying mutans streptococcus. The mutans when implanted, remain localized to the site of implantation and does not spread easily to other areas. Colonisation of new surface does not occur until the salivary count of mutans streptococcus reaches a critical value

of about 4.5 x 10^4 per ml for smooth surface and about 10^3 for fissure caries. The mutans streptococcus have a localized way of colonising the dentition, which mean that some tooth surface may have mutans and others are not. The amount of mutan streptococcus in saliva is related to the number of colonised surfaces. This fact is the basis for saliva test for mutans streptopcoccus[18].

Alaluusua S, Myllarniemi S, Kallio M (1989) [2] evaluated the level of Streptococcus mutans in stimulated saliva and its association with caries experience in 149 5-year-old children. In general, salivary S. mutans levels were low, and it was detected only in 46% of saliva samples. There was, however, a clear association between salivary levels of S. mutans and caries experience. Salivary examination was supplemented with plaque samples in 47 children. The number of S. mutans positive surfaces increased with increasing salivary levels. S. mutans was most often isolated and comprised the highest proportion in the approximal samples. The number of children with high salivary S. mutans levels was very low (6%) when taken into account that 13% of the children were fairly caries active (dmfs $\geq$ 5). This most probably means that in evaluation of caries risk, the salivary S. mutans screening level is different in preschool children and in older children. The level should be determined in longitudinal studies before applying to preschool children.

Ali Y.A, Chandranee N.J, Wadher B.J, et al (1998) [4] evaluated the relationship between caries status, colony forming units (cfu) of streptococcus mutans and Snyder caries activity test. The streptococcus mutans were isolated

using MSB agar and cfu were determined of each individual from different caries groups. The saliva of the same individual was drooled into a culture bottle containing Snyder test agar and extent of colour change was observed after 24, 48, and 72 hours incubated at 37^{o}C. The time and extent of colour change determined the conduciveness of diet. It has been observed that caries free individuals have low cfu count and their diet was mild or moderately conducive, while the individuals having average caries had medium cfu count and their diet was moderate-highly conducive. In rampant caries, individuals however the cfu count were high and the diet was highly conducive. In caries free, average caries and rampant caries group 40, 60, and 80 per cent samples showed colour change after 72 hrs with Snyder test. Thus a definite correlation exists between caries status, cfu count of streptococcus mutans and Snyder caries activity test.

Chopra S, Taneja J.R, Rai J, et al (1994) [9] studied the microflora in well-nourished and mal-nourished children in relation to dental caries. Forty five children in the age group of 2-12 years comprising 20 well-nourished (W.N.), 20 mal-nourished (M.N.) (both groups having DMFS of $\geq$ 5) and 5 caries free well nourished children (control group) were studied to find out the oral microflora in these groups S. mutans was present in 55%, 20% and 0% in W.N., M.N., and control groups while S. Salivarius was isolated in 45%, 80% and 40% in the three groups respectively. There was no significant difference in the prevalence of Lactobacillus and C. albicans in the three groups. The co-relation between mean DMFS and the prevalence of S. mutans in W.N. and

M.N. groups was highly significant ($P < 0.001$). Nutritional status including different grades of malnutrition had no significant bearing on the prevalence of micro-organisms isolated. The caries prevalence was higher in the W.N. group (P) while the gingival index was higher in M.N. group.

Crall J.J, Edelstein B, Tinanoff N, et al (1990) [11] investigated the significance of social, environmental, and biological variables in relation to caries status in a group of young children, and to determine whether incorporating data on social and environmental variables into a multivariate model could improve the accuracy of a screening approach that relies solely on quantifying levels of salivary Streptococcus mutans. Data regarding fluoride status and sociodemographic characteristics were collected from the dental records of 89 children who ranged in age from 10-71 months, and who had been screened previously for S. mutans. Multivariate analyses (logit) revealed that the probability of having clinically or radiographically detectable caries was associated with 1) higher levels of salivary S. mutans, 2) residing in a single-parent household, 3) having suboptimal levels of fluoride in the drinking water and 4) not being covered by a dental insurance plan. The findings attest to the importance of considering social and environmental factors, in addition to biological variables, when evaluating caries status in young children.

Dasanayake A.P, Caufield P.W, Cutter G.R, et al (1993) [13] conducted a randomized clinical trial to determine the transmission of mutans streptococci to infants following short term application of an iodine-NaF solution to

mothers dentition. Six applications of an I-NaF or a placebo solution were administered to the mothers' dentition at the time of tooth emergence of her infant, to evaluate its effect on transmission of mutans streptococci (MS) to the infant. MS and other bacterial levels were periodically monitored in 48 mother-infant pairs until the child's third birthday. There were significant reductions in maternal salivary MS (p = 0.04), lactobacilli (P = 0.04), total streptococci (P = 0.002), and total cultivable organisms (P = 0.004) immediately following treatment. In children, 3-yr incidence of MS colonization and the time of acquisition of MS or the caries experience did not differ significantly between the two groups. We conclude that short-term application of I_2-NaF to the maternal dentition of predentate infants significantly lowers MS, lactobacilli, total streptococci, and total cultivable bacteria in mothers' saliva, but does not influence the incidence and the time of acquisition of MS or the caries experience in children.

Fujiwara T, Sasada E, Mima N, et al (1991) [16] evaluated the prevalence of caries and the number and species distribution of salivary mutans streptococci in 356 children (aged 0-2 yr old) in Japan were examined twice at an interval of 1 yr. No mutans streptococci were isolated from the predentate children. The detection rate of mutans streptococci and the prevalence of caries increased with age. Mutans streptococci were isolated from 39.9% of the subjects. The majority of isolates were identified as Streptococcus mutans. The concentration of mutans streptococci correlated with the number of erupted teeth (r = 0.339). In addition, the concentration of mutans streptococci was

more closely correlated to the caries prevalence of the next year (r = 0.465) than that of the year when the salivary concentration of mutans streptococci was first evaluated (r = 0.368). The children who had no caries but harbored mutans streptococci in the first year showed significantly higher caries prevalence in the next year than did the children with neither caries nor mutans streptococci. These results indicate that the establishment of mutans streptococci is associated with caries initiation in early childhood.

Gomez I, Rio D, et al (1991) [17] evaluated dental caries and mutans streptococci in selected groups of urban and native Indian schoolchildren in Mexico. Two groups of Mexican children aged 12-14 yr were examined for dental caries and salivary mutans streptococci counts. One hundred lived in Mexico City and 100 belonged to a native Mazahua Indian population. The prevalence of caries, diagnosed according to WHO, was 90% in Mexico City and 82% in the Indian community. Mean DMFT was 5.98 and 3.57 respectively. Saliva samples were analyzed for mutans streptococci by the "Strip mutans" method. Further identification of the biotype/serotype was done. Mutans streptococci were found in 95% of the urban children and 75% of the Indians. There was a statistically significant difference in the prevalence of mutans streptococci between the city and the rural samples. Streptococcus mutans was more frequently found than Streptococcus sobrinus.

Klock B, Svanberg M, Petersson L.G, et al (1990) [24] evaluated the dental caries, mutans streptococci, lactobacilli, and saliva secretion rate in

adults. In 718 Swedish patients, equally divided into four age groups (19-25, 26-45, 46-60, > 60 yr), salivary levels of mutans streptococci and lactobacilli, saliva secretion rate, and DMFS were registered. No significant differences were found between the various age groups either in salivary factors or in caries (D). Number of missing (M) and filled (F) surfaces increased with age. Prevalence of root caries, which increased with age, was significantly correlated to number of exposed root surfaces independent of age. Of the total study group, 50% had $\geq 10^6$ mutans streptococci and 40% had $\geq 10^5$ lactobacilli per mL saliva. Three percent had a saliva secretion rate of $\geq$ 0.5 mL/min. Correlation analyses showed that both mutans streptococci and lactobacilli significantly correlated to the caries prevalence but the r-value never exceeded 0.34.

Krishnakumar R, Singh S, Subba Reddy V.V, et al (2002) [27] compared the levels of mutans streptococci and lactobacilli in children with nursing bottle caries, rampant caries, healthy children with 3-5 dmft/DMFT and healthy caries free children. The children from each group of nursing caries, rampant caries, 3-5 dmft/DMFT and caries free children were selected and plaque samples from carious lesion, early carious lesion, sound tooth surface as well as salivary sample were collected. The levels of mutans streptococci and lactobacilli were estimated at all the sample sites. In all the groups, the mean levels of mutans streptococci were significantly higher than lactobacilli in early carious lesions and on the sound tooth. In nursing caries group, the mean levels of mutans streptococci and lactobacilli were significantly higher from the carious lesions

when compared to early carious lesions and sound tooth surface. The result was similar in rampant caries group. In 3-5 dmft/DMFT, the streptococci level was significantly higher at both, carious lesions and at early carious lesion while lactobacilli level was higher at carious lesion. Salivary mutans streptococcal levels were significantly higher than lactobacilli in the group. Surprisingly 4 individuals of caries free group showed high levels of mutans streptococci and 6 individuals showed moderate levels.

In nursing caries, rampant caries and 3-5 dmft/DMFT groups, both mutans streptococci and lactobacilli were in high counts at the carious lesion, while in early carious lesion and sound tooth, the mutans streptococci exhibited higher counts than lactobacilli. In the caries free group, the mutans streptococci counts were higher than lactobacilli in the plaque samples. The findings provide further support for the role of mutans streptococci in the initiation of human dental caries and role of lactobacilli in its progression.

Purohit V.D, Damle S.G, (1996) [35] estimated the salivary counts of mutans streptococci, lactobacilli, flow rate and buffering capacity in caries free and caries active children. The study comprised 120 healthy children (61 boys & 59 girls) in the age group of 12-24 months (mean age 12-27 years) from 5 municipal schools in Mumbai Central area. A total of 120 children comprising 40 with no detectable caries and 80 with caries were randomly assigned to 4 different groups. Each group included 30 children (10 with no detectable caries and 20 with caries).

The tests carried out were:

a) Salivary flow rate

b) Salivary buffering capacity (final pH)

c) Salivary lactobacilli count and

d) Salivary mutans streptococci count.

Children with NDC (Group V) showed lower mean flow rate than that in Group 1 (1.66 ml/min.) and 1.7 ml/min.). However, mean buffering capacity was higher than the corresponding value in Group II (6.84 and 6.73 pH respectively). Both microbial counts in this group were conspicuously lower than the mean counts in group III and IV. A positive correlation was found only between the flow rate and buffering capacity (r = 0.496) in this group. In conclusion, it can be said that the children with NDC exhibited higher buffering capacity and lower microbial counts than those in children with caries. While evaluating individual susceptibility to caries, a single test may not be helpful and several parameters considered together can be of immense help. The correlations found with the microbial counts were promising. This study can serve as a basis to develop a caries prediction model in the future.

Rusell J.I, MacFarlane T.W, Aitchison T.C, et al (1990) [39] compared caries prevalence and microbiological and salivary caries activity tests in Scottish adolescents. Salivary and microbiological caries activity tests were investigated on three occasions in a group of 372 Scottish adolescents. Counts of lactobacilli, mutans streptococci, and candida were consistently and

significantly associated with caries prevalence, as either Ds or DMFS score, and buffering capacity was consistently inversely related to DMFS score. However, veillonella counts and salivary flow rate were not correlated with caries prevalence. Significant improvements in the associations were obtained when the results of more than one test were included using stepwise regression analysis. On an individual basis, at most, stepwise discriminant analysis identified the DMFS group correctly in 49% of all subjects, and the DS group in 47%.

Steiner M, Helfenstein U, Menghini G, et al (1998) [43] presented an epidemiologic study aimed at detecting a possible association of salivary mutans streptococci with brown discoloured pits and fissures, supposing that discolouration indicates caries. In the Canton of Zurich 1035 schoolchildren, aged 6.5 – 12.5, were examined with regard to caries prevalence and presence of discolourations in pits and fissures. A commercially available, semi-quantitative test was used to estimate the salivary level of mutans streptococci in each child. The salivary level (low/high) of mutans streptococci was significantly associated with the presence of slightly brown discoloured (C1), clearly brown discoloured (C2) and cavitated (C3) pits and fissures. The odds ratios were 1.5 ($P<0.01$) for C1, 2.5 ($P<0.001$) for C2 and 5.0 ($P<0.001$) for C3 pits and fissures. The findings are consistent with the hypothesis that brown discolouration indicates caries. Furthermore, the findings suggested that this type of discolouration at elementary school age indicates increased caries activity.

Suhasini K, Reddy C.D, Hamid S.A, et al (1997) [44] did a comparative evaluation of salivary Streptococcus mutans levels in children with rampant caries and their mothers salivary levels of Streptococcus mutans in fifty children with rampant caries in the age group of 3-6 years and their mothers were compared with fifty caries free children of the same age groups and their mothers. Significantly higher levels of Streptococcus mutans were seen in children with rampant caries and also in their mothers revealing the possible transmission of Streptococcus mutans from mother to child.

Sullivan A, Borgstrom M.K, Granath L, et al (1996) [45] considered the saliva counts to be a reasonable indicator of the entire dentition's total microbial load. However, the value of salivary counts for explaining and predicting caries have been found to be low. There was therefore reason to compare the relationships between caries on the one hand and, on the other, the number of MS or LB in plaque and in saliva, respectively, in order to assess their relative merits for explaining the variation in caries, both in a total material and in subgroups with less favourable oral hygiene. Sixty children aged 14-15 years participated in the study. Caries and plaque were registered and the number of MS and LB was estimated in total plaque and in stimulated saliva samples. The results showed that the number of MS or LB in plaque did not explain the variation in caries to a greater degree than did the salivary counts.

Thibodeau E.A, O'Sullivan D.M, Tinanoff N, et al (1993) [48] evaluated the relationship between salivary mutans streptococci levels and caries in preschool children of low socioeconomic status. A total of 462 Head Start children, mean age 3.8 yr (range 2.0-5.3 yr), were examined by the modified method of RADIKE. Saliva samples from 458 of these children were collected with tongue blades and impressed onto mutans streptococci selective agar. Children's mutans streptococci levels were categorized as low (0 CFU), moderate (1-50 CFU) or high (> 50 CFU) and the mean dmfs was 0.40, 1.92 and 4.88, respectively. All study groups (Black, Hispanic and White) had infection rates of approximatey 83%; however, 39.1% of Black children had high mutans streptococci levels compared with 28.4% of White children. Pit/fissure caries was the most prevalent disease type in children with moderate or high mutans streptococci levels, although White children in the high group had significantly less of this pattern than Blacks and Hispanics. Sensitivity, specificity, and positive and negative predictive values for the high mutans streptococci group were 91.3%, 57.5%, 69.3% and 86.3%. Results from the current study suggested a direct relationship between mutans streptococci levels and mean dmfs, with increased levels of infection associated with increased dmfs. The strength of this association is greater in Black and Hispanic children than in White children. Thus for our population, those children who are Black or Hispanic and have high levels of salivary mutans streptococci can be categorized as having the highest level of caries risk.

Thibodeau E.A, O'Sullivan D.M, Tinanoff N, et al (1993) [49] evaluated the correlation between mutans streptococci levels and dental caries. The aim of this study was to assess the relationship between salivary mutans streptococci levels and caries in preschool children of low socioeconomic status. A total of 462 Head Start children, mean age 3.8 yr (range 2.0 – 5.3 yr), were examined by the modified method of RADIKE. Saliva samples from 458 of these children were collected with tongue blades and impressed onto mutans streptococci selective agar. Children's mutans streptococci levels were categorized as low (o CFU), moderate (1-50 CFU) or high (> 50 CFU), and the mean dmfs was 0.40, 1.92 and 4.88, respectively. All study groups (Black, Hispanic and White) had infection rates of approximately 83%; however, 39.1% of Black children had high mutans streptococci levels compared with 28.4% of White children. Pit/fissure caries was the most prevalent disease type in children with moderate or high mutans streptococci levels, although White children in the high group had significantly less of this pattern than Blacks and Hispanics. Sensitivity, specificity, and positive and negative predictive values for the high mutans streptococci group were 91.3%, 57.5%, 69.3% and 86.3%. Results from this study indicate that differences between Black, Hispanic and White preschool children may influence caries activity within populations that have similar mutans streptococci infection levels and socioeconomic backgrounds.

Thibodeau E.A, Sullivan D.M, (1995) [50] assessed the relationship between salivary mutans streptococcus (SMS) levels and the prevalence and

incidence of dental caries in 148 children (mean baseline age 3.8 years) of low socioeconomic status. Caries data (dmfs) and semiquantitative SMS counts were obtained at baseline and annually for 2 years. The children were classified during each of the 3 years as low (0 colony-forming units; CFU), moderate (1-50 CFU), or high (>50 CFU) caries risk based on total SMS counts. The results indicated that the prevalence of dental caries increased with SMS levels at baseline and generally in both assessment year. The results indicate that there is an association between SMS levels and prevalence and incidence of dental caries in the primary teeth of these children and suggest that SMS levels may be useful in predicting the caries risk in the deciduous dentition of some prpulations.

Thibodeau E.A, O'Sullivan D.M, et al (1996) [51] assessed the association between levels of salivary mutans streptococci and the prevalence, incidence and distribution of caries patterns in the primary dentition. A cohart of pre-school children (n = 146, mean age 3.8 yr) were examined for dental caries and sampled for salivary mutans streptococci (SMS) at baseline and once annually for 2 yr. Children's tooth surfaces were categorized into four patterns: pit/fissure, maxillary anterior, posterior proximal, and buccal/lingual smooth surface. Salivary mutans streptococci were enumerated using a tongue blade technique, and were categorized as low (0 CFU), moderate (1-50 CFU) and high (> 50 CFU). At year 2, children with high baseline SMS had the 1) highest prevalence of caries (87%) and the highest dmfs (9.15); 2) highest prevalence of each pattern, and 3) greatest number of patterns. Among children with the

pit/fissure pattern, those with high baseline SMS had the greatest pit/fissure dmfs after 2 yr.

The results suggested that in this population, children with low baseline salivary mutans streptococci levels are more likely to be caries-free and have a lower dmfs after 2 yr than children with moderate and high baseline levels. It showed a positive association between salivary mutans streptococci levels and the prevalence of caries patterns.

Thibodeau E.A, O'Sullivan D.M, et al (1999) [52] examined the relationship between SMS and longitudinal caries development in the primary and mixed dentitions. Eighty-five children, initial mean age 3.8 years, were examined for dental caries at baseline and once annually for 6 years. Children's SMS were sampled with a tongue blade, which was impressed onto plates containing a medium selective for SMS. After incubation, colony forming units of SMS were determined semi-quantitatively and categorized as low, moderate or high. Children classified as high caries risk at baseline had significantly greater ($P<0.05$) dmfs scores for all teeth, and in the primary molars, than children classified as moderate or low caries risk at every age but 9 ($P<0.10$). Children classified as high risk at age 3 had the greatest DMFS increment through age 8. Based on annual examinations, there was a trend towards increasing mean dmfs/DMFS scores among children classified as high risk in every year.

The current study is among the first to report on the ability of annual measurements of SMS to identify long-term caries risk in both the primary and the mixed dentitions. Despite limitations in predicting caries risk using microbiological methods, this longitudinal study supports the overall benefits of this type of testing.

Weinberger S.J, Wright G.Z, et al (1990) [54] assessed the reliability of the tongue depressor for saliva sampling, and whether the sampling time during the day influences S. mutans counts. The study population consisted of 27 children, between 24 and 66 months of age. Samples of unstimulated saliva were gathered on tongue blades four times during the day for each subject. Each child was scheduled for paired saliva samples at the following times: 1) within a half hr after breakfast, 2) mid-morning, having last eaten 1 to 1-1/2 hr previously 3) before lunch with 1 hr elapsing since a mid-morning snack, and 4) following lunch and an afternoon nap, 1-1/2 hr since the last meal. Paired samples representing both sides of the tongue blades were inoculated onto elevated agar plates containing a selective medium, and anaerobically incubated at 37oC for 48 hr. After examining 76 paired samples of saliva, no significant differences in s. mutans counts were found between sides of the spatula, suggesting that the sampling technique was suitable. Analysis of variance showed significant differences within each subject over the four sampling times ($P < .01$).

In the mid morning-after lunch comparison, 13 subjects increased S. mutans counts after lunch, four were the same and five subjects counts decreased. In the before lunch vs. after lunch comparison, nine subjects S. mutans counts increased, 10 were the same, and six decreased. There was a borderline difference between after-breakfast and mid-morning, which might be beyond chance if the sample had been larger. In conclusion, if food intake can influence S. mutans counts, a suggestion is that children should not eat, and drinking should be limited to water 1-1/2 to 2 hr before a caries activity test is performed.

The salivary non-microbial parameters have clinical relevance in maintaining the healthy and balance, consequently, whose assays have clinical applications in particular to evaluate the activity and susceptibility of dental caries in either individuals or populations. Salivary flow rate is undoubtedly the most important single parameter since the cariostatic activity or efficacy of practically all other salivary parameters depends on the flow rate. Flow rate as such has no linear association with dental caries but there seems to exist an individual “threshold” limit which is decisive for enhanced caries activity. This threshold limit varies among different individuals and therefore the so-called normal values for unstimulated or stimulated flow rate are more reliable on a population level than among individuals for screening purposes. In any individual a regular and longitudinal follow-up of the flow rate is of higher clinical value than only a single cross-sectional measurement. Salivary buffer effect has only a weak negative association with caries activity and again, this

effect is of greater clinical significance on a population level. Since the decisive processes in caries attack occur within or under the dental plaque, the buffering effect of saliva is limited and obviously more important to screen the caries-prone individuals [47].

Caries in early childhood has been considered a 'dieto-bacterial disease'. Bowen maintains that 'diet influences the expression of virulence but may also affect the intrinsic virulence of cariogenic microorganisms'. Dietary sugars, particularly sucrose, are the main dietary factors in caries. There is a dose-response relationship between dietary sugars and caries. Hence increase in frequency of sugar consumption has been associated with an increase in dental caries.

Akpata E.S, Shammery A.R, Saeed H.I, et al (1992) [1] evaluated the dental caries, sugar consumption and restorative dental care in 12-13-year-old children in Riyadh, Saudi Arabia. A 3-day dietary history was obtained from 363 Riyadh schoolchildren aged 12-13 yr, after which their dentitions were examined for dental caries. About 16-31% of the children were caries-free and the mean DMFT varied between 1.67 and 2.43. However, in those with at least one tooth decayed, missing or filled, the mean DMFT remained constant at about 3, irrespective of age or gender; and most of the carious teeth were unrestored. There was a statistically significant relationship between DFS and the frequency of sugar consumption on the first 2 days of the dietary diary. It

was suggested that the management of dental caries in the children must include the control of dietary sugar.

Anderson P, Hector M.p, Rampersad M.A, et al (2001) [5] compared the critical pH in resting and stimulated whole saliva in group of children and adults. In this comparative study, calcium and phosphate concentrations was measured in the resting and stimulated saliva of child and adult volunteers using a spectrophotometric system used in routine blood analysis. Salivary flow rates were measured in each group. The calcium concentrations were lower in children than adults, but the phosphate concentrations were not significantly different. The critical pH significantly higher for children than adults in both resting and stimulated saliva.

Bretz W.A, Djahjah C, Almeida R.S, et al (1992) [7] evaluated the relationship of microbial and salivary parameters with dental caries in Brazilian pre-school children. Caries examination and collection of paraffin wax-stimulated saliva samples were performed in 37 children, 3-6 years old, in a child-care facility at the Vidigal slum, Rio de Janeiro, Brazil. The levels of mutans streptococci and lactobacilli in saliva were estimated by the Cariescreen and by the Dentocult tests and the saliva secretion rate was determined. Statistical analysis was performed on surface-based and patient-based caries prevalence rates (SBCPR and PBCPR), and related to bacterial and salivary parameters. The results show that 31 of the 37 children were caries active. The SBCPR for the primary dentition was 6.7% ± 1.0%. Occlusal surfaces were the

most affected by decay. Regression analysis revealed that mutans streptococci salivary levels were significantly associated with the SBCPR (P=0.0001). Similarly, lactobacilli salivary levels were significantly associated with the SBCPR (P=0.0001). No significant association could be found between the saliva secretion rate ad the SBCPR. When regression analysis was used to model dependence of the SBCPR on both organisms, the mutans streptococci and lactobacilli salivary levels were significantly associated with the SBCPRs (P=0.0021 and 0.0118, respectively) and salivary levels of these organisms accounted for 57% of the SBCPR variability. These findings indicated that the levels of mutans streptococci and lactobacilli in saliva are significantly related to the SBCPRs on the primary dentition of these children.

Dan Ericson, Bratthall D, et al (1989) [12] measured the buffer capacity of stimulated saliva samples from 62 subjects with the new method. This method, Dentobuff Strip, consists of a pH indicator paper that has been impregnated with acid. A small volume of saliva is added to the strip and after 5 min the color of the strip is compared with a chart. The colors have been chosen to indicate low, medium, or good buffer capacity. This method was compared with two commonly used clinical methods, Dentobuff, and an electrometric method. The three methods correlated well, and patients with a low salivary buffer capacity (final pH $\leq$ 4), according to the electrometric method, were readily identified with the new Dentobuff Strip method. The Dentobuff Strip method was found to be a reliable method and simpler to use

than previous methods for identifying subjects with low or very low buffer capacity.

Holbrook W.P, Kristinsson M.J, Gunnarsdottir S, et al (1989) [23] evaluated the caries prevalence, streptococcus mutans and sugar intake among 4-year-old urban children in Iceland. Concern at the high prevalence of dental caries in Iceland prompted this survey of 158 preschool children in Reykjavik. On initial examination in 1987 45.8% of the children were caries-free and the prevalence of caries was a mean dmft 2.4 and a mean dmfs 3.3. A dietary questionnaire aimed at discovering the frequency of sugar consumption per week revealed a threshold value of 30 instances of sugar intake per week above which caries prevalence increased markedly. Some of this sugar is consumed in paediatric medicines, particularly antibiotics and anti-asthmatics, which are widely used in Iceland. Caries prevalence (dmft) for children taking such medicines regularly was 3.0 compared with 2.1 for remaining children ($P<0.05$). Those children who took fluoride tablets regularly had less caries (mean dmft 1.1) than those who used fluoride tablets irregularly or not at all (mean dmft 2.8; $P<0.001$). Samples of saliva from the dorsum of the tongue were taken for determination of numbers of Streptococcus mutans and lactobacilli. High bacterial counts were strongly associated with caries. Only 5% of children with more than 5×10^5 S. Mutans cfu per ml were caries free but 27% of subjects had 67% of the total amount of caries for the group and all of these would have been detected by the bacterial test.

Maciel S.M, Marcenes W, Sheiham A, et al (2001) [30] analysed the relationship between preference for sweetness, levels of salivary mutans streptococci and levels of caries in low socio-economic 4-5 years-old Brazillian children. 298 children of both sexes, who in 1998, were regularly attending public nurseries were randomly selected. Caries experience was assessed according to WHO guidelines. Saliva samples were analysed for mutans streptococci using the spatula method. Children's preference for sugar was measured using the 'Sweet Preference Inventory'. Personal interviews with the mothers were conducted. 255 children completed all aspects of the research, a response rate of 85.6%. 34.9% of them were caries-free. The mean dmfs was 4.25 (SD 6.16), the decayed component being 3.56 (SD 5.82) and the filled surfaces, 0.69 (SD 1.85). For these pre-school children; male sex ($P<0.01$), single parent ($P<0.01$), habit of eating or drinking items that contained non-milk extrinsic sugar between meal times ($P<0.05$) and high levels of salivary mutans streptococci ($P< 0.001$) were significantly associated with higher dmfs scores. There was no statistical relationship between sweetness preference and dental caries and with mutans streptococci levels.

Levels of salivary mutans streptococci but not sweetness preference were potential good predictors of caries experience among 4-5 years-old-children living in urban area of Brazil.

Mazengo C.M, Tenovno J, Hausen H, et al (1996) [31] assessed the relationship between diet and dental caries in a Tanzanian population was

studied. Mutans streptococci, lactobacilli, yeasts, salivary flow rate as well as buffer effect were also analyzed. A random sample of 12-,35-44 and 65-to 74 year olds was drawn from Msongola (rural) and Ukombozi (urban), Dar-es-Salaam. The mean of two 24-h recalls was sued for the assessment of food intake. The percentage of those with at least one carious tooth ranged from 30% in the 12 year olds to 80% in the oldest age group. The mean number of decayed teeth (DT) increased significantly with age (P=0.000) but was not significantly associated with the area of residence. DT increased significantly (P=0.048) with the number of snacks per day and was also associated with dietary sucrose (P=0.025), total carbohydrates (P=0.002) and fiber (P=0.002). Among salivary variables lactobacilli (P=0.000) correlated positively with DT. Our study did not reveal any strong association between total energy intake and dental caries in rural or urban populations in Tanzania but snacking and sucrose intake were significantly associated with caries, in particular in the urban area.

Rodrigues C.S, Sheiham A, et al (2000) [36] investigated the effects of dietary guidelines on sugar and sugar intake at day nurseries, and other potential risk factors on dental caries in two groups of low socio-economic nursery schoolchildren. The study population comprised 510, 3-year-old low socio-economic nursery schoolchildren. Sugar intake at the nursery was based upon a weighed inventory method during 2 non-consecutive days. Parents answered a questionnaire. Sugar intake at nursery and the adoption of guidelines on sugar were associated with lower caries increment in low income nursery schoolchildren. A number of modifiable factors such as a higher daily

frequency and weight of sugar intake at nursery, use of fluoride and habits related to tooth brushing were strongly related to caries increment.

Seppa L, Pollanen L, Hausen H, et al (1988) [41] examined the relationship of Streptococcus mutans counts obtained by a Dip-Slide Method to Caries Frequency, Sucrose Intake and Flow Rate of Saliva the level of Streptococcus mutans in saliva was determined by a dip-slide method in 841 13 – yr old children in order to identify children with high caries risk. For each child, the flow rate of saliva was determined. Caries scores were obtained from Public Dental Health records. A sucrose intake score was calculated based on self-reported frequency of intake of six types of sugary products. As S.mutans counts increased, there was a significant trend of increased DMFS and DS scores. No linear correlation was observed between ported intake of sucrose and S.muans counts, but the children with the highest counts (class 3) tended to have significant higher sucrose intake than the rest of the children. The flow rate of saliva decreased significant as S.mutans increased.

Szpunar S.M, Eklund S.A, Burt B.A, et al (1995) [46] assessed the risk from sugar consumption in a population of schoolchildren with low caries experience. It relates eight different measures of sugar consumption to the occurrence of any DMFS increment, and, separately, to approximal and pit-and-fissure DMFS. The data are from a 3-yr longitudinal study of 429 children, initially aged 11-15, residing in non-fluoridated rural communities in Michigan, USA. All children completed at least three dietary interviews, were

present for baseline and final dental examinations, and had a parent or guardian provide questionnaire information on residence history, use of fluoride and dental services, and family history. Results indicated that a higher proportion of total energy intake from sugars increased the probability of caries on all surfaces, and a higher total intake of sugars was also associated with total caries increment. No relationship, however, was found between DMFS increment and the frequency of eating high sugar foods. Each additional 5 g of daily sugars intake was associated with a 1% increase in the probability of developing caries, and those whose energy intake from sugars was 1 SD above the mean had 2.0 times the risk of developing approximal caries than did children whose energy intake from sugars was 1 SD below the mean.

Tukia-Kulmala H et al 1993 [21] evaluated the intra-and inter-individual variation in salivary flow rate, buffer effect, and the levels of salivary mutans streptococci and lactobacilli were analyzed in 128 11-year-old children. The follow-up period was 9 months, with six saliva samplings done at regular intervals. Inter-individual variation was relatively large in paraffin-stimulated salivary flow rate: (<1.0ml/min) and high (≥ 2.0ml/min) flow rates were measured in 18% and 13% of the children, respectively. Intraindividual variation during the follow-up period was found in 63% of the boys and in 73% of the girls. The buffer effect stayed stable in all samplings in 59% of the boys and in 42% of the girls. Buffer effect was significantly ($p< 0.001$) lower in girls than in boys. Mutans streptococci were analyzed by a chair-side method (Strip mutans test) and by cultivation on mitis-salivarious-bacitracin (MSB) agar

plates. The results of the two methods correlated highly significantly ($r = 0.79$. $p < 0.001$). With the Strip mutans test no variation in test scores occurred in 49% of all subjects in all six samplings, whereas the respective percentage for MSB scores was only 19%. No variation in salivary lactobacilli occurred in only 18% of the subjects, and in 13% the intraindividual variation was as high as ≥ 3 logs. In the present study, intra-individual variability of buffer effect was much greater in girls than in boys. The large variability in bacterial counts is obviously considerably influenced by gradually exfoliating primary teeth with concomitant emergence of new tooth surfaces at the age of 11-12 years. Our observations emphasize that, in line with the bacterial tests, measurements of buffer effect and flow rate are highly variable at this age. Therefore, only single point measurements of salivary variables should not be used for caries-diagnostic or predictive purposes for children with a developing dentition.

Materials & Methodology

MATERIALS & METHODOLOGY

The present study comprised of 100 healthy children in the age group of 3-5 years from local nursery schools in Chennai. All children were in the primary dentition stage. A proforma was prepared to record the data.

Children with the history of antibiotic consumption within last 3 months or were receiving any other antimicrobial agents concurrent with this study period, fluoride use or living in fluoridated area, any prior dental treatment were not included in the study. This was possible to study the natural occurrence of dental decay that was unclouded by the effects of treatment and preventive measures [7].

MATERIALS:

Materials used for the study were given below.

Interview

Questionnaires in English.

Oral examination

1. Hand gloves.
2. Mouth mask.
3. Mouth mirror.
4. Explorer or Ash's number 54 probe.
5. 2x2 gauze piece.

Biochemical study

1. Sugarless chewing gums.
2. Sterile disposable saliva collecting cups.
3. Pipette.
4. Stop watch.
5. pH indicator paper strips with colour coded chart.

Microbiological study

1. Inoculating loops.
2. Pipette.
3. Conical flasks.
4. Sterilizer.
5. Distilled water.
6. Sterile Petri dish.
7. Refrigerator.
8. Incubator.
9. Candle jar.
10. Hand magnifying lens.
11. Microscope.
12. Microscopic glass slide**.**

Chemicals and solutions

1. Mitis salivarius bacitracin agar medium.
2. Saline.
3. Gention violet
4. Gram's iodine.
5. Absolute alcohol.
6. Dilute carbon fusion
7. Sorbitol.
8. Mannitol.
9. Starch.

PROFORMA

GENERAL INFORMATION

1. Name : _____
2. Age/ Sex : _____
3. Address : _____

_____

MEDICAL HISTORY

1. Did you take any antibiotic for the past 3-6 months ? : (a) Yes (b) No

2. Are you on any medication now? : (a) Yes (b) No

If "yes" in what form? : (a) Syrup (b) Tablet

DENTAL HISTORY

1. Have you undergone any dental treatment before? : (a) Yes (b) No

If "yes" : (a) Extraction
(b) Filling
(c) Others

PERSONAL HISTORY

1. Brushing habits

Device : (a) Finger (b) Neemstick (c) Brush (d) Others
Material : (a) Powder (b) Paste (c) Others
Frequency : _____ / day

2. Any other fluoride supplements in use? : (a) Yes (b) No

If "yes" in which form? : (a) Tablet (b) Solution (c) Gel

DIETARY QUESTIONNAIRE

I WEEK DIETARY QUESTIONNAIRE								
QUESTIONNAIRE		FREQUENCY OF SUGAR INTAKE						
		Mon	Tues	Wed	Thur	Fri	Sat	Sun
1.	Do you take any sugar containing foods between meals?							
2.	Do you take any sugar containing foods at night?							
3.	Do you take any sugar containing foods after brushing?							
	"SUGAR INDEX"							

"dft" INDEX

55	54	53	52	51	61	62	63	64	65

85	**84**	**83**	**82**	**81**	**71**	**72**	**73**	**74**	**75**

d =_____

+ =

f = _____

SCORE (dft) =

BIOCHEMICAL TEST

1. Salivary (stimulated) Flow Rate =_____ / min
2. Salivary Buffering capacity P_H = _____

MICRO BIOLOGICAL EVALUATION

Mutans streptococcus counts = _____ cfu

RECORDING OF DENTAL CARIES:

Dental examination was carried out on a chair , under natural lighting conditions. The teeth were wiped with a 2x2 gauze piece. The diagnosis of dental caries was predominantly by the visual methods and this was augmented by the gentle use of explorer or Ash's number 54 probe to remove dental plaque and confirm the softness of caries lesions and the caries experienced was assessed in the entire mouth with the dft index**. (Grubbel 1944).** This value gave the caries status of the individual.

CRITERIA FOR EVALUATION OF DENTAL CARIES:

d=number of primary teeth present that are carious

f=Number of filled primary teeth.

t=Number of teeth involved.

Children were divided into two categories on the basis of their caries experience;

1. Children with no clinically detectable caries or caries free.

2. Children with caries.

Group 1:50 children with no detectable caries.

Group 2:50 children with caries.

Children in caries group are categorized in to three grades depending on the number of decayed tooth.

Grade 1 : 1 – 3 decayed teeth

Grade 2 : 3 – 5

Grade 3 : > 5

DIETARY QUESTIONNAIRE:

Parents were asked to attend with their child in the school and at that time they were questioned closely about the child's dietary habits. A questionnaire was specifically designed which sought to discover the frequency of sugar consumption rather than the total amount of sugar eaten.

CRITERIA FOR EVALUATION OF DIETARY QUESTIONNAIRE:

Parents were asked what the child ate at meal times and between meals on weekdays and at weekends. Particular attention was placed on determining the nature of sweetening agents in the soft drinks consumed and the use of sugar containing foods between meals and at night or after the teeth had been brushed. From the completed questionnaire**,** it was possible to calculate the number of occasions that sugar was consumed with meals, snacks, sweets and drinks, To this figure, calculated for 5 week days, was added the number of occasions sugar was similarly consumed at week ends and the total for 1 week termed as "sugar index" for the child was determined [23].

PROCEDURES:

The salivary samples were collected from the subjects on second visits. The tests were carried out using mid-morning (9 am-11 am) saliva samples. The investigations of ***Weinberger et al (1990)*** [55] showed that any food ingestion can alter the salivary physiochemical properties as well as microbial load. Therefore the salivary samples were collected at least 1.5 hrs-2hrs after the breakfast.

1. Salivary flow rate:

The secretion of whole mixed saliva was measured after stimulation. Prior to stimulation the subjects were asked to eliminate any existing saliva in the mouth. Each child was given a sugarless chewing gum and was instructed to chew on both sides. Stimulated secretion was collected in sterile saliva collecting cups. Collection was tried for a period of 5 minutes. Since the composition of saliva depends on the duration of stimulation, the saliva collected during the first minute has a different composition from saliva collected after 5 minutes of constant stimulation. For example, concentration of calcium and protein will increase during prolonged stimulation [6]. The foam was excluded by using the graduated plastic cylinder since the foam usually does not contain significant amount of salivary secretion. Then salivary flow rate was measured and was calculated per minute [47].

Stimulated salivary flow rate expressed in ml/min			
	Hypo salivation	Low	Normal
Stimulated saliva	Grade 1 = < 0.7	Grade 2 = 0.7-1	Grade 3 = >1

2. Salivary buffering capacity (pH):

Stimulated whole mixed saliva was used to determine buffering capacity. The estimation was carried out using a pH indicator paper that had been impregnated with acid. A small volume of saliva was taken with the pipette from the salivary samples and added to the strips. After 5 minutes the colour of the strips were compared with the colour coded chart which had

numerical value. The colours had been chosen to indicate low, medium or good buffering capacity [21].

pH	Inference
Grade 1 = ≤4	Low
Grade 2 = 4.5-5.5	Medium
Grade 3 = ≥6	High

3. Salivary microbiological tests:

The collected samples were inoculated on Mitis salivarius bacitracin agar medium (MSB agar), which is a highly selective medium for S.mutans, for counting colony-forming units (C.F.U).

Preparation of mitis salivarius bacitracin agar (MSB) medium

Contains tryptose -Peptone agar 0.01 gm/ml, 20% sucrose, 75 µg/ml tryphan blue, 0.8 µg/ml crystal violet, 10 µg/ml potassium tellurite, 0.2 units/ml bacitracin.

8.7 gms of mitis salivarius agar medium were added to 1 liter of distilled water and soaked for 15 minutes. The solution was sterilized by autoclaving for 15 minutes at 15 lb per square inch (121^{o}C). Then it was cooled to 50 ^{o}C and 0.1ml of 1% potassium tellurite was added. To this 0.2 units/ml bacitracin was added.

It was well mixed and poured into sterile petri dishes. The principle advantage of MSB agar is that it allows the isolation of the species even when present in low numbers relative to the total populations.

The selectivity of the media had been achieved by raising the sucrose concentration in the original media (MS) from 5 to 20 % and addition of bacitracin. Sucrose allows s.mutans to form colonies with the characteristic morphology which facilitates it identification, while the other micro flora are suppressed by high sucrose concentration [40]. Bacitracin inhibited other oral streptococci but not mutans [22]. St.salivarians which forms large slimy colonies that can overgrow the plate, is inhibited by bacitracin[40].

On MSB agar, s.mutans grow as highly convex to pulvinate (cushion shaped) colonies, which are irregular in shape.

None of the strains of s.sanguis.s.mitis,s.salivarius,s.milleri grow on MSB agar except s.mutans.

Culture methods:

A sterile inoculating loop of 10 μl interval diameter was inserted vertically into the sample and loopful of sample was picked up and streaked over the surface of culture plate as follows for counting C.F.U.

1. Loop was touched to the centre of the agar plate from which the inoculum was spread in a line across the diameter of the plate.
2. Without flaming or re-entering, the sample loop was drawn across the entire plate crossing the first inoculum streak numerous times to produce isolated colonies.

Recording Colony Forming Units (cfu) count:

The isolated plates of Mitis salivarius bacitracin agar was incubated at 37 ^{o}C in a candle jar environment for 48 hours. After 2 days the number of s.mutans were counted and recorded semi quantitatively as Grade 0 = '0' (no detectable cfu), Grade 1 = 1-9 cfu (Low), Grade 2 = 10-100 cfu (Medium) and Grade 3 = > 100 cfu (High) and the results were tabulated.

Confirmatory tests:

Confirmation of s.mutans was done by

1. Smear examination.
2. Biochemical tests.

Smear examination:

1. The plate was made from the colonies grown on MSB agar plates on a clean glass slide (about half an inch circle) air dried and fixed by passing through flame 3 times.
2. The heat fixed smear was primarily stained with 1% gentian violet for 1 minute.
3. Then the stain was washed away with distilled water and 1% gram's iodine was put for 1 minute.
4. Gram's iodine was thrown away and the smear decolorized with 3% acid alcohol until the colour of the smear turned faint.
5. Finally the smear was counted and stained with dilute carbol fuschin for 30 seconds.

6. The smear was washed with distilled water, air-dried and examined under the oil immersion 100% objective.

Biochemical tests:

S.mutans gave positive results with manniol and sorbitol and negative with starch.

Colonies from the MSB agar plate were picked up with sterile inoculating loops and inoculated into the test tubes containing various serum sugars. The inoculated sugar tubes were incubated at 37 ^{o}C for 48 hrs. Colour changes indicated the fermentation of the sugars by the organisms.

ARMAMENTARIUM USED FOR THE STUDY

Fig. 1 Armamentarium for clinical examination

Fig . 2 Clinical examination

Fig . 3 Saliva collecting sterile disposable cups

Fig . 4 Collection of Stimulated salivary sample

Fig . 5 Determination of Salivary buffering capacity

Fig . 6 Armamentarium for MSB Agar media preparation

Fig.7 Weighing machine

Fig 8.Incubator

Fig 9 Autoclave

Fig 10 Laminar airflow

Fig11.Armamentarium for Gram staining

Results

RESULTS

The present study was carried out to evaluate the relation between caries experience and sugar intake, salivary flow rate, salivary buffering capacity, salivary mutans streptococcus count and the possible inter relationship between these variables in 50 caries and 50 caries free, 3 – 5 years old preschool children.

The analysis was done using chi-square test and the results are as follows:

Observations from Table 1

Table 1 showed mean values of dft, sugar index, salivary flow rate, salivary buffering capacity, and salivary mutans streptococcus count of test and control group.

The mean values of dft in test and control group were 4.98 $\pm$2.21 and 0.00 $\pm$ 0.00 respectively. The mean values of sugar index in test and control group were 37.62 $\pm$ 6.25 and 26.42 $\pm$1.95 respectively. The mean values of salivary flow rate in test and control group were 1.01 $\pm$ 0.27 and 1.50 $\pm$ 0.29 respectively. The mean values of salivary buffering capacity in test and control group were 5.87 $\pm$ 0.57 and 7.00 $\pm$ 0.29 respectively. The mean values of salivary mutans streptococcus count in test and control group were 72.14 $\pm$ 43.31 and 0.98 $\pm$ 0.85 respectively.

Observations from Table 2

Table 2 showed the distribution of test and control group according to dft, sugar index, salivary flow rate, salivary buffering capacity and salivary mutans streptococcus counts.

When distributed according to their sugar intake, 18 children (36%) in test group and 50 children (100%) in control group belonged to Grade 1 sugar index. 32 children (64%) in test group belonged to Grade 2 sugar index. The test group showed increased frequency of sugar consumption whereas the control group had decreased frequency of sugar consumption.

When distributed according to their caries status, 50 children (100%) in control group belonged to Grade 0. 15 children (30%) in test group belonged to Grade 1. 15 children (30%) in test group belonged to Grade 2. 20 children (40%) in test group belonged to Grade 3.

When distributed according to their salivary flow rate level, 3 children (6%) in test group belonged to Grade 1. 26 children (52%) in test group and 1 child (20%) in control group belonged to Grade 2. 21 children (42.0%) in test group and 49 children (98%) in control group belonged to Grade 3. The test group showed more tendency to have low flow rate whereas the control group had normal or high flow rate.

When distributed according to their salivary buffering capacity, none of the children belonged to Grade 1. 27 children (54%) in test group belonged to

Grade 2. 23 children (46%) in test group and 50 children (100%) in control group belonged to Grade 3. The test group showed more tendency to have medium buffering capacity, whereas the control group had high buffering capacity.

When distributed according to their mutans streptococcus count in saliva, 16 children (32%) in control group belonged to Grade 0. 7 children (14%) in test group and 34 children (68%) in control group belonged to Grade 1. 20 children (40%) in test group belonged to Grade 2. 23 children (43%) in test group belonged to Grade 3. The test group had highest mutans count whereas, the control group had lowest or no mutans count.

Observations from Table 3

Table 3 showed the relationship of caries status Graded from 0 – 3 with the sugar index Graded as 1 and 2.

Based on the caries status, 50 children (73.5%) in Grade 0, 15 children (22.1%) in Grade 1, 3 children (14.4%) in Grade 3 belonged to Grade 1 sugar index. 12 children (37.5%) in Grade 2, 20 children (62.5%) in Grade 3 belonged to Grade 2 sugar index. As the frequency of sugar consumption increased the caries experience also increased. There was a statistically significant relationhip between frequency of sugar consumption and caries experience in the children corresponding to the p-value of 0.001.

Observations from Table 4

Table 4 showed the relationship between caries status Graded from 0 – 3 and the salivary flow rate Grade from 1 – 3.

Based on the caries status 3 children (100%) in Grade 3 belonged to Grade 1 flow rate. 1 child (3.7%) in Grade 0, 2 children (7.4%) in Grade 1, 7 children (25.9%) in Grade 2, 17 children (63%) in Grade 3 belonged to Grade 2 salivary flow rate. 49 children (70%) in Grade 0, 13 children (18.6%) in grade 1, 8 children (11.4%) in Grade 2 belonged to Grade 3 salivary flow rate. As the flow rate decreases, there was an increase in caries experience. There was a statistically significant relation between flow rate and caries experience with the p-value of 0.001.

Based on the caries status 2 children (7.4%) in Grade 1, 8 children (29.6%) in Grade 2, 17 children (63.0%) in Grade 3 belonged to Grade 2 buffering capacity. 50 children (68.5%) in Grade 0, 13 children (17.8%) in Grade 1, 7 children (9.6%) in Grade 2, 3 children (4.1%) in Grade 3 belonged to Grade 3 buffering capacity. As the buffering capacity decreases, there was an increase in caries experience. There was a statistically significant relation between buffering capacity and caries experience with the p-value of 0.001.

Observations from Table 5

Table 5 showed the relationship between caries status Graded from 0 – 3 and salivary mutans streptococcus count Graded from 0 – 3.

Based on the caries status, 16 children (100%) in Grade 0 belonged to Grade 0 mutans count. 34 children (82.9%) in Grade 0, 7 children (17.1%) in Grade 1 belonged to Grade 1 mutans count. 8 children (40%) in Grade 1, 8 children (40%) in Grade 2 and 4 children (20%) in Grade 3 belonged to Grade 2 mutans count. 7 children (30.4%) in Grade 2, 16 children (69.6%) in Grade 3 belonged to Grade 3 mutans count. As the mutans count increases, there was a significant increase in caries experience. There was a statistically significant relation between streptococcus mutans count and caries experience with the p-value of 0.001.

Observations from Table 6

Table 6 showed the relationship of salivary mutans streptococcus count Graded from 0 – 3 with the sugar index Graded as 1 and 2.

Based on the mutans streptococcus count in saliva, 16 children (23.5) in Grade 0, 41 children (60.3%) in Grade 1, 9 children (13.2%) in Grade 2, 2 children (2.9%) in Grade 3 belonged to Grade 1 sugar index. As the frequency of sugar consumption decreases, there was a significant decrease in mutans count. There was a statistically significant relationship between < 32 frequency

of sugar consumption and mutans streptococcus count in saliva with a p-value of 0.001.

Based on the mutans streptococcus count in saliva, 11 children (34.4%) in Grade 2 and 21 children (65.6%) in Grade 3 belonged to Grade 2 sugar index. The relationship between increased frequency of sugar consumption (Grade 2 = > 32) and mutans streptococcus count in saliva was weak with a p-value of 0.07, but the children with the highest mutans count (Grade 3) had higher sugar intake than those in remaining three Grades.

Observations from Table 7

Table 7 showed the relationship between mutans streptococcus count in saliva Graded from 0 – 3 and the salivary flow rate Graded from 1 – 3.

Based on the mutans count in saliva, 3 children (100%) in Grade 3 belonged to Grade 1 flow rate. None of the children with the mutans count of Grade 0, 1,2 belonged to Grade 1 flow rate. There was a statistically significant relation between mutans count in saliva and Grade 1 salivary flow rate with a p-value of 0.001. 1 child (3.7%) in Grade 1 mutans count belonged to Grade 2 salivary flow rate. As the flow rate decreases, there was an increase in the number of mutans count in saliva. There was a statistically significant relationship between salivary mutans streptococcus count and Grade 2 (Low) salivary flow rate with the p-value of 0.001. 16 children (22.9%) in Grade 0, 40 children (57.1%) in Grade 3, 13 children (18.6%) in Grade 2, 1 child (1.4%) in

Grade 3 belonged to Grade 3 flow rate. There was a statistically significant relationship between mutans count and Grade 3 flow rate. The children with lowest mutans count belonged to Grade 3 i.e. had normal salivary flow rate.

Observations from Table 8

Table 8 showed the relationship between mutans streptococcus count in saliva Graded from 0 – 3 and the salivary buffering capacity Graded from 1 – 3.

None of the children belonged to Grade 1 buffering capacity. 8 children (29.6%) in Grade 2 mutans count, 19 children (70.4%) in Grade 3 belonged to Grade 2 buffering capacity. As the buffering capacity in saliva decreases, there was an increase in mutans count in saliva. There was a statistically significant relationship between medium buffering capacity and mutans streptococcus count in saliva with a p-value of 0.03. 16 children (21.9%) in Grade 0, 41 children (56.2%) in Grade 1, 12 children (16.4%) in Grade 2, 4 children (5.5%) in Grade 3 mutans count belonged to Grade 3 buffering capacity. As the buffering capacity increases, there was a decrease in mutans count in saliva. There was a statistically significant relationship between normal buffering capacity and mutans streptococcus in saliva with a p-value of 0.001.

Observations from Table 9

Table 9 showed the relationship between salivary flow rate Graded from 1 – 3 and the salivary buffering capacity Graded from 1 – 3.

Based on the buffering capacity in saliva 3 children (100%) in Grade 2 belonged to Grade 1 salivary flow rate. 22 children (81.5%) in Grade 2, 5 children (18.5%) in Grade 3 belonged to Grade 2 salivary flow rate. As the flow rate decreases, the buffering capacity in saliva also decreased. There was a statistically significant relationship between low flow rate and buffering capacity with the p-value of 0.001. 2 children (2.9%) in Grade 2 and 68 children (97.1%) in Grade 3 belonged to Grade 3 buffering capacity. As the flow rate increases, there was an increase in buffering capacity of saliva. There was a statistically significant relationship between normal flow rate and buffering capacity with a p-value of 0.001.

Table 1

MEAN VALUES OF CARIES STATUS, SUGAR INDEX, SALIVARY AND MICROBIAL PARAMETERS OF CARIES GROUP (TEST GROUP) AND CARIES FREE GROUP (CONTROL GROUP)

Variables	Caries Group (Test Group)	Caries Free Group (Control Group)
	Mean	Mean
dft	4.98 ±2.21	0.00 ± 0.00
Sugar Index	37.62 ± 6.25	26.42 ± 1.95
Stimulated Salivary Flow Rate	1.01 ± 0.27	1.50 ± 0.29
Salivary Buffering Capacity	5.87 ± 0.51	7.00 ± 0.29
Salivary Mutans Streptococcus Count	72.14 ± 43.31	0.98 ± 0.85

Table 2

DISTRIBUTION OF TEST AND CONTROL GROUP ACCORDING TO CARIES STATUS, SUGAR INDEX, SALIVARY FLOW RATE, SALIVARY BUFFERING CAPACITY AND SALIVARY MUTANS STREPTOCOCCUS COUNT

Variables	Grade	Test Group		Control Group	
		No.	%	No.	%
dft	0	0	0	50	100
	1	15	30.0	0	0
	2	15	30.0	0	0
	3	20	40.0	0	0
Sugar Index	1	18	36.0	50	100
	2	32	64.0	0	0
Salivary Flow Rate	1	3	6.0	0	0
	2	26	52.0	1	2.0
	3	21	42.0	49	98.0
Salivary Buffering Capacity	1	0	0	0	0
	2	27	54.0	0	0
	3	23	46.0	50	100.0
Salivary Mutans Streptococcus Count	0	0	0	16	32.0
	1	7	14.0	34	68.0
	2	20	40.0	0	0
	3	23	46.0	0	0

dft in Grades

Grade 0 = 0	No clinically visible caries
Grade 1 = 1 – 3	decayed teeth
Grade 2 = 3 – 5	decayed teeth
Grade 3 = > 5	decayed teeth

Sugar Index in Grades

Grade 1 = < 32	Frequency of sugar consumption
Grade 2 = > 32	Frequency of sugar consumption

Stimulated Salivary Flow Rate in Grades

Grade 1 = < 0.7 ml / min	Hyposalivation
Grade 2 = 0.7 – 1ml/min	Low
Grade 3 = > 1ml / min	Normal

Salivary Buffering Capacity in Grades

Grade 1 = $\leq$ 4	Low
Grade 2 = 4.5 – 5.5	Medium
Grade 3 = $\geq$ 6	High

Salivary Mutans Streptococcus count in Grades

Grade 0 = 0	No detectable c.f.u
Grade 1 = 1 – 9	Low
Grade 2 = 10 – 100	Medium
Grade 3 = > 100	High

Table – 3

RELATIONSHIP BETWEEN CARIES STATUS AND SUGAR INDEX

Sugar Index in Grades	dft in Grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
1	50	73.5	15	22.1	3	4.4	0	0	88.97	<0.001***
2	0	0	0	0	12	37.5	20	62.5		

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

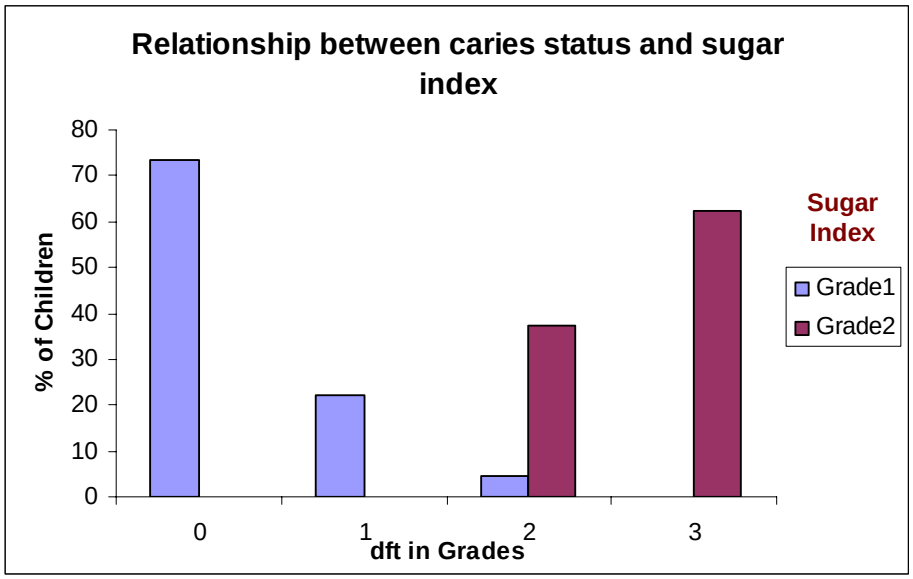


Table – 4

RELATIONSHIP BETWEEN CARIES STATUS AND SALIVARY PARAMETERS

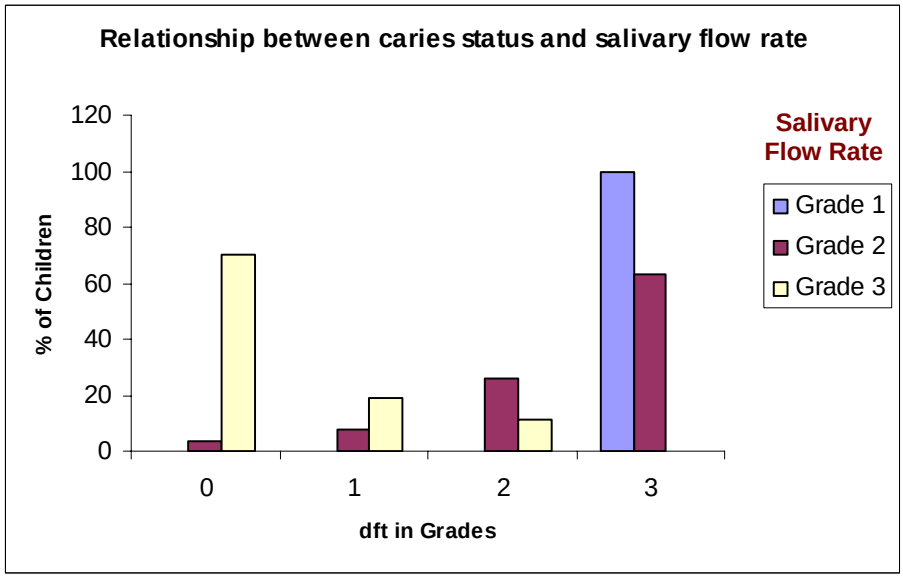
Variables in Grade		dft in Grades								Chi-Square Value	P-Value
		0		1		2		3			
		No.	%	No.	%	No.	%	No.	%		
Salivary Flow Rate	1	0	0	0	0	0	0	3	100.0	72.47	<0.001***
	2	1	3.7	2	7.4	7	25.9	17	63.0		
	3	49	70.0	13	18.6	8	11.4	0	0		
Salivary Buffering Capacity	1	0	0	0	0	0	0	0	0	59.33	<0.001***
	2	0	0	2	7.4	8	29.6	17	63.0		
	3	50	68.5	13	17.8	7	9.6	3	4.1		

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%


Relationship between caries status and salivary flow rate
120
100
80
60
40
20
0
% of Children
0
1
2
3
dft in Grades
Salivary Flow Rate
Grade 1
Grade 2
Grade 3

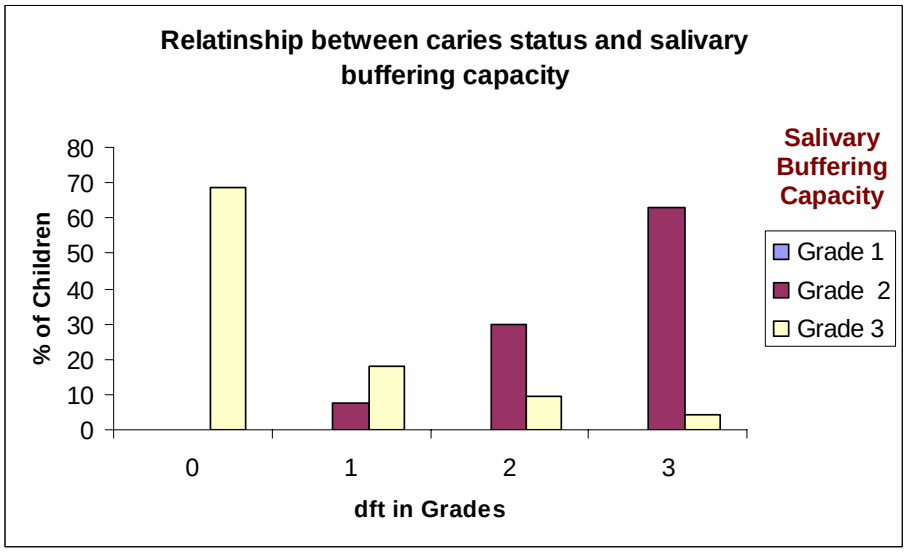

Relatinship between caries status and salivary buffering capacity
80
70
60
50
40
30
20
10
0
% of Children
0
1
2
3
dft in Grades
Salivary Buffering Capacity
Grade 1
Grade 2
Grade 3

Table – 5

RELATIONSHIP BETWEEN CARIES STATUS AND SALIVARY MUTANS STREPTOCOCCUS COUNT

Salivary mutans Streptococcus counts in Grade	dft in Grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
0	16	100.0	0	0	0	0	0	0	112.88	<0.001***
1	34	82.9	7	17.1	0	0	0	0		
2	0	0	8	40.0	8	40.0	4	20.0		
3	0	0	0	0	7	30.4	16	69.6		

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

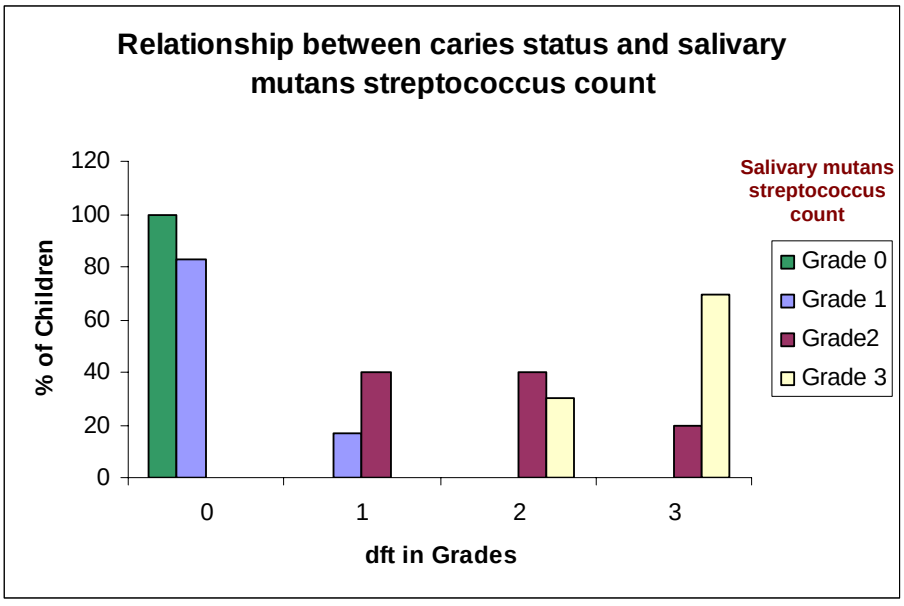


Table – 6

RELATIONSHIP BETWEEN MICROBIAL PARAMETER AND SUGAR INDEX

Sugar index in Grades	Salivary mutans streptococcus counts in grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
1	16	23.5	41	60.3	9	13.2	2	2.9	50.942	0.001***
2	0	0	0	0	11	34.4	21	65.6	3.1250	0.07 NS

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

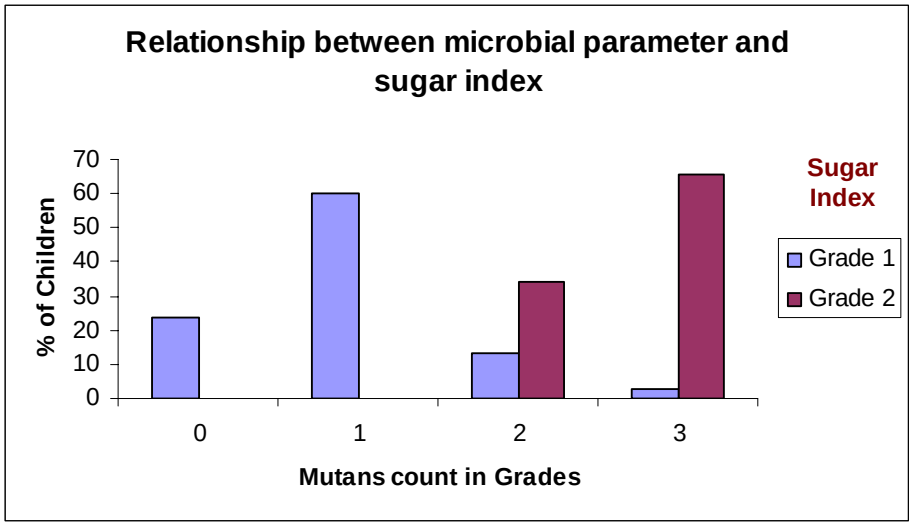


Table – 7

RELATIONSHIP BETWEEN MICROBIAL PARAMETER AND SALIVARY FLOW RATE

Salivary flow rate in Grades	Salivary Mutans Streptococcus Count in Grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
1	0	0	0	0	0	0	3	100.0	17.647	<0.001***
2	0	0	1	3.7	7	25.9	19	70.4	18.667	0.001***
3	16	22.9	40	57.1	13	18.6	1	1.4	45.77	<0.001***

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

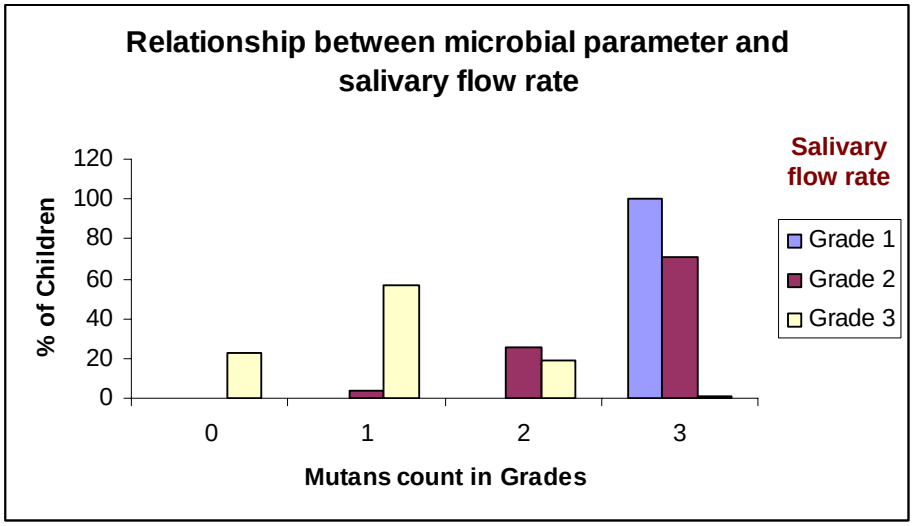


Table – 8

RELATIONSHIP BETWEEN MICROBIAL PARAMETER AND SALIVARY BUFFERING CAPACITY

Salivary Buffering capacity in Grades	Salivary Mutans Streptococcus Count in Grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
1	0	0	0	0	0	0	0	0	0.000	<0.001***
2	0	0	0	0	8	29.6	19	70.4	4.48	0.030*
3	16	21.9	41	56.2	12	16.4	4	5.5	41.90	0.001***

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

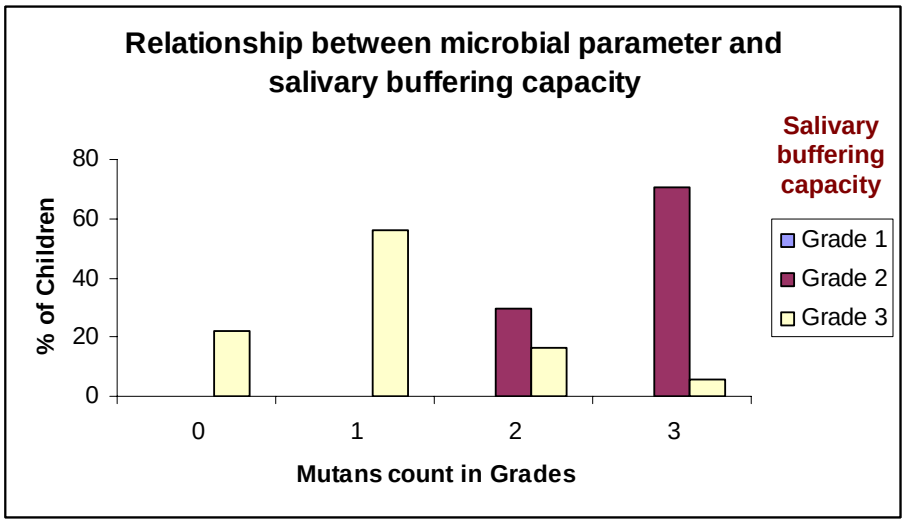


Table – 9

RELATIONSHIP BETWEEN SALIVARY FLOW RATE AND SALIVARY BUFFERING CAPACITY

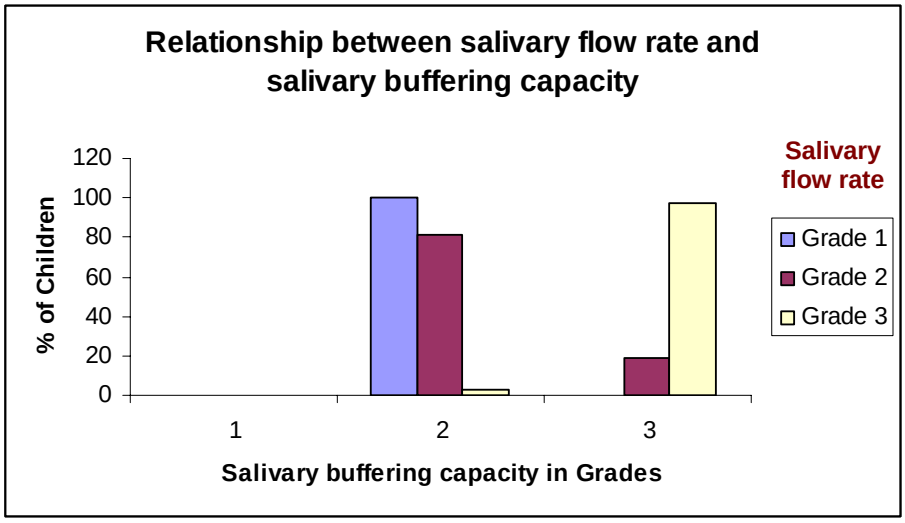
Salivary Flow rate in Grades	Salivary Buffering capacity in Grades						Chi-Square Value	P-Value
	1		2		3			
	No.	%	No.	%	No.	%		
1	0	0	3	100.0	0	0	17.647	0.001***
2	0	0	22	81.5	5	18.5	10.70	0.001***
3	0	0	2	2.9	68	97.1	62.23	0.001***

NS = Not significant

* = P < 0.05 significant at 5%

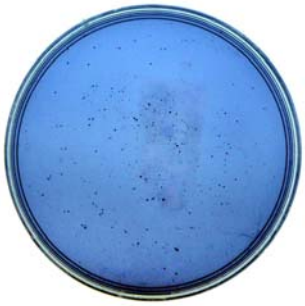
** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

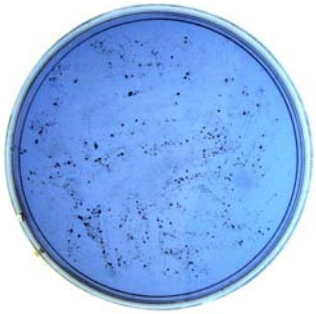


GRADE: 0

GRADE: 1



GRADE: 2



GRADE: 3

Fig. 12. Colony forming units of mutans streptococcus graded from 0- 3

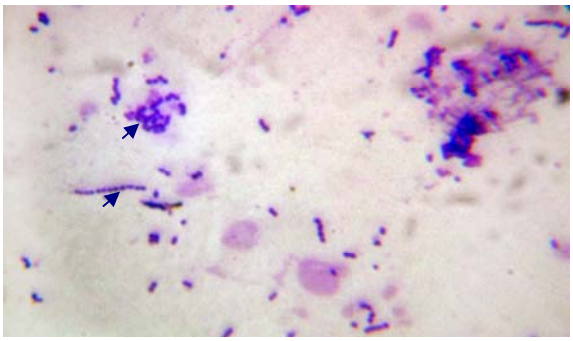


Fig 13.Microscopic view of mutans streptococcus

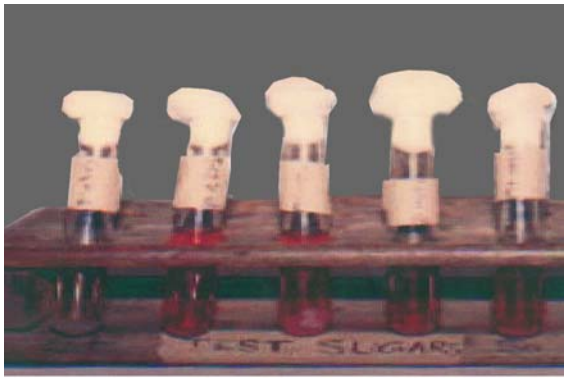


Fig 14. Bio chemical test – mutans streptococcus reaction to starch, mannitol and sorbitol

Discussion

DISCUSSION

The present study included 100 children. 50 caries children in test group and 50 caries free children in control group. The age group presented in this study was 3-5 years. As this age group presented with their primary dentition could be of interest, so as to control the level of cariogenic organisms at this early age, so that the upcoming dentition would not be challenged by the cariogenic ability of these organisms. The present study was designed to evaluate four variables i.e. Sugar Index, Salivary flow rate, Salivary buffering capacity and Salivary streptococcus mutans count and its relationship with the caries experience and the relationship within the four variables of 3-5 year old preschool children.

The earlier models to represent the key factors involved in dental caries was put forth by *Fitzgerald and Keyes*. Recently, the concept of the caries balance was first published by *Featherstone in 1999* [15] in an attempt to simplify the key factors involved in dental caries progression or reversal and make them readily applicable in clinical practice and easily understandable for the patient. The three pathological factors are cariogenic bacteria, fermentable carbohydrates and saliva dysfunction and the protective factors are saliva components and salivary flow, fluoride and antibacterial therapy. The above three pathological factors and three protective factors are not exclusive, but are the most important factors in assessing future caries risk and identifying why a person has caries or can be expected to show continuous caries progression. The 3-vs-3 concept should be the first step in providing a risk assessment and

an understanding of the caries process as it is progressing in an individual patient. As per the concept of Featherstone, the caries experience tend to be related to microbial, non-microbial salivary parameters *(Tenovuo et al 1997)* [47] and frequency of sugar intake *(Bjarnason et al 1982, Newbroun et al 1978).* In the extensive field of caries research, various studies have been done to correlate the relation between these potent etiological factors and the caries experience *(Bretz 1992* [7]*, Holbrook et al 1989* [23]*, Purohit et al 1996* [35] *).*

The current study focused on the relation of these variables with the caries experience and the possible inter-relationship if any between these variables among 3 – 5 year old preschool children.

The children in the present study were graded from 0 – 3 (Grade 0 = 0 (no decayed teeth), Grade 1 = 1 – 3 (decayed teeth), Grade 2 = 3 – 5, Grade 3 = > 5) based on the caries status (dft). The dietary questionnaire was taken, particular attention was placed on determining the use of sugar containing foods between meals and at night or after the teeth had been brushed. From the completed questionnaire, it was possible to calculate the number of occasions the sugar was consumed for 1 week termed as "sugar index" for the child [30]. The sugar index was graded as Grade 1 (< 32 frequency of sugar consumption) and Grade 2 (> 32 frequency of sugar consumption).

The stimulated salivary samples were collected to determine the flow rate, buffering capacity and mutans count. The caries process occurs during and immediately following ingestion of cariogenic foods. It is precisely during

this period, that the saliva is stimulated. This would suggest that stimulated rather than resting saliva should be studied [19]. Stimulated salivary sample was collected for period of 5 minutes in a sterile saliva collecting cups. Then the secretion was measured and flow rate / min was calculated. The salivary flow rate was graded from 1 – 3 (Grade 1 = < 0.7 ml / min (Hyposalivation), Grade 2 = 0.7 – 1 ml (low) Grade 3 = > 1 ml / min (normal))[47]. The estimation of buffering capacity was carried out using a pH indicator paper strips with the colour coded charts and graded from 1 – 3 (Grade 1 = $\leq$ 4 (Low), Grade 2 = 4.5 – 5.5 (Medium), Grade 3 = $\geq$ 6 (High))[21]. To determine the mutans count the salivary samples were inoculated on the MSB agar medium and the colony forming units of streptococcus mutans were counted and graded from 0 – 3 (Grade 0 = 0 (no c.f.u), Grade 1 = 1 – 9 c.f.u, Grade 2 = 10 – 100 c.f.u, Grade 3 = > 100 c.f.u).

The results (Table 3) from the present study indicated that 73.5% of the children who were caries free had < 32 frequency of sugar consumption. 62.5% of children with Grade 3 dft had > 32 frequency of sugar consumption. The children with increased frequency of sugar intake (>32) showed a rise in dft and children with decreased frequency of sugar intake (<32) were caries free. As mentioned in the other studies by *Holbrook et al 1989* [23], *Akapta et al 1992* [1], *Mazengo et al 1996* [31], there was a statistically significant relationship between frequency of sugar consumption and caries experience ($p < 0.001$). The etiologic role of diet, particularly that of fermentable carbohydrates, in dental caries is well accepted. The presence of sucrose and other fermentable

carbohydrates favour colonization of the dentition by acidogenic microorganisms *(Rolla 1985* [38]*, Loesche 1986* [29]). Although there is no question that the ingestion of sugar-containing foods is necessary for caries to occur, it is also true that certain characteristics of such foods. and the circumstances under which they are eaten are more important determining their cariogenic potential than the amount of sugar they contain. Perhaps the most conclusive proof to this effect was provided by the now classical *Vipeholm Study (1946 – 1950).* The frequency with which cariogenic foods are eaten are more important. The more frequently, the more cariogenic they become. Additional evidence concerning the enhancement of cariogenic activity by the frequent ingestion of sugar-containing foods was provided by *Weiss and Trithart (1960).*

The current study (Table 4) showed 100% of children in Grade 3 dft had hyposalivation, 63% of children in Grade 3 dft had low flow rate, 70% of children who were caries free had normal flow rate. 63% of children in Grade 3 dft had medium buffering capacity and 68.5% of children who were caries free had high buffering capacity. The children with decreased flow rate and buffering capacity showed a rise in dft and the children who were caries free had normal flow rate and high buffering capacity. Thus the relationship between salivary parameters and caries experience were statistically highly significant ($p < 0.001$). The results of the present study correlated with the other studies that have examined the relationship of salivary parameters on caries experience and the inverse relation between salivary parameters and caries experience. *(Dan Ericsson et al 1989*[12]*, Purohit et al 1996* [35]*).* The saliva

has a number of various functions, obviously the most important one is the clearance of oral micro-organisms and food components from the mouth to the gut. This balance can be disturbed either by extensive growth of bacteria – as a consequence of for example, poor oral hygiene, abundant use of fermentable carbohydrates or some systemic diseases – or by reduced salivary flow rate which in turn results in increase caries attack. The flow of the whole saliva is of clinical relevance for the susceptibility and activity of dental caries [47].

The results (Table 5) from the current study showed 100% of children who were caries free had no detectable mutans count, 69.6% of children in Grade 3 dft had highest mutans count (Grade 3). This suggested a direct relationship between mutans streptococci level in saliva and dft, with increase levels of mutans streptococci associated with increased dft and no mutans in caries free group ($p < 0.001$). The relationship between mutans streptococci and caries experience had been reported for several population of preschool children. Typically, the mean dft and dfs have been shown to increase with the increasing levels of mutans streptococci. *(Chosack et al 1988* [10]*, Holbrook et al 1989* [23]*, Alalussua et al 1989* [2]*, Weinberger et al 1989* [55]*).* The amount of mutans streptococci in saliva is related to number of colonized surfaces on the teeth. The highest mutans streptocci levels may predict high-risk children at an early age. *Alaluusua and Renken 1982* [2]*, Kohler and colleagues 1988* [25]*, Fujiwara et al 1991* [16]*, Roeters et al 1995* [37], clearly demonstrated that early infection with mutans streptococci is a significant risk factor for future development of dental caries. Hence, because of the widely accepted

etiological role of mutans streptococci in dental caries, the term "Early childhood caries" is recently accepted in the terminology of "Maternally Derived Streptococcus Mutans Disease" (MDSMD).

The results (Table 6) from the present study indicated a weak relation, between sugar intake and mutans streptococcus count ($p = 0.07$), but the children with the highest mutans count (Grade 3) had a higher sugar intake (Grade 2) than those remaining three Grades. Lack of significant relationship between mutans count and sucrose intake was in agreement with the results of *Hargreaves et al 1980* [20] *and Tofferson et al 1986*. According to *Sheiham 1987* [42] *and Heldermann et al 1996*, the increase in number of mutans streptococci and their correlation to dental caries is to a larger extent dependent on diet. However, the association between the habit of ingesting sugary foods and levels of salivary mutans streptococci was not statistically significant *(Maciel et al 2001)* [30].

The present study (Table 7) showed three children with the highest mutans count (Grade 3) had hyposalivation (Grade 1). 19 children with highest mutans count (Grade 3) and 7 children with medium mutans count (Grade 2) had low flow rate (Grade 2). As the flow rate decreases, there was a significant increase in mutans count ($p < 0.001$). 13 children with the medium mutans count (Grade 2), children with the lowest mutans count (Grade 1) and children with no mutans in saliva had normal flow rate (Grade 3). As the flow rate increases there was a significant decrease in mutans count ($p < 0.001$). The

strong relation between mutans count and salivary flow rate was in accordance with the other studies that had evaluated the effect of flow rate on mutans count in saliva *(Tukia et al 1993* [21]*, Seppa et al 1998* [41]*).* The relation between mutans count and salivary flow rate seems to be important. A rapid flow rate of saliva inhibit the growth of mutans streptococci through antibacterial systems of saliva or by rapid elimination of carbohydrates from the mouth [47].

In the present study (Table 8), none of the children had low buffering capacity. 19 children with the highest mutans count (Grade 3) and 8 children with the medium count (Grade 2) had medium buffering capacity (Grade 2). As the buffering capacity decreases there was a significant increase in mutans count ($p = 0.03$). 41children with the lowest mutans count (Grade 1), 12 children with the medium count (Grade 2) and children with no mutans count (Grade 0) in saliva had higher buffering capacity (Grade 1). As the buffering capacity increases there was a significant reduction in mutans count ($p < 0.001$). The relation between salivary buffering capacity and mutans count indicated low or medium buffer effect showed poor salivary resistance against microbial attack. *(Lagerlof et al 1994)* [28]. Among these individuals the clearance of micro-organisms is slow.

The present study (Table 9) showed 3 children with the medium buffering capacity (Grade 2) had hyposalivation (Grade 1). 22 children with the medium buffering capacity (Grade 2) had low flow rate (Grade 2). As the flow rate decreases there was a significant decrease in buffering capacity ($p <$

0.001). Children with high buffering capacity had normal or high flow rate. As the flow rate increases there was a significant increase in buffering capacity in saliva ($p < 0.001$). There was a statistically significant relation between flow rate and buffering capacity that correlated with the other studies by *Tukia et al 1993* [21]. The most important buffering system in stimulated saliva is carbonic acid / bicarbonate system. The concentration of bicarbonate ions varies from the less than 1mM in unstimulated saliva to 60mM at high flow rate. Hence there is a strong relation between salivary flow rate and buffering capacity [6].

So from this study it is observed that there was a significant relation between caries experience and salivary flow rate, buffering capacity, salivary streptococcus mutans count, sugar intake. Streptococcus mutans count correlated reasonably well with the flow rate and buffering capacity but to a lesser extent with the reported sugar intake. The salivary flow rate and buffering capacity had a direct relationship with each other. This multifactorial approach of identifying high caries risk group children and implementation of preventive strategies represents an improvement over an approach that relied solely on single parameter. The present study demonstrated the importance of considering sugar intake, microbial and non microbial salivary parameters when assessing the caries activity in children. Although strong evidence exists that these parameters affect the caries process, on an individual or population basis they offer only little predictive value. Therefore future research should focus on exploring combinations, or clusters of various parameters which are typical for caries active and inactive individuals. Instead of measuring

individual salivary parameters, it is likely that some functional measures of saliva could provide a better relationship with caries activity is needed. Hence future research should focus on the other reliable parameters that can be incorporated to predict the caries status of the individual.

Conclusion

CONCLUSION

The present study was undertaken in the local nursery schools of Chennai among 3 – 5 year old preschool children to evaluate the sugar intake, the microbial and nonmicrobial parameters and its relation with the caries experience and if any interrelationship between these variables.

The following conclusion were drawn from the study:

1. There was a highly significant relation between frequency of sugar consumption and caries experience. ($P < 0.001$)
2. There was a inverse relation between salivary parameters (salivary flow rate, buffering capacity) and the caries experience. ($P < 0.001$)
3. There was a direct relation between microbial parameter (salivary mutans streptococcus count) and the caries experience. ($P < 0.001$)
4. There was a strong relation between salivary parameters (salivary flow rate, buffering capacity) and the streptococcus mutans count. ($P < 0.001$)
5. There was a weak relation between frequency of sugar consumption and the streptococcus mutans count. ($P = 0.07$)
6. There was a direct relation between salivary flow rate and salivary buffering capacity. ($P < 0.001$)

The need to search for risk indicators for dental caries in order to target risk groups has been one of the subjects of a recent conference on the assessment of risk group in dentistry. These parameters evaluated in the current study could be used to discriminate highly infected subjects from non

infected subjects and therefore may be indicators of an increased or reduced risk for dental decay. After an initial diagnosis of risk indicators, the high risk groups are identified and therapy should be directed to preventive strategies such as the use of fluorides, sealants and other antimicrobial supplements. Further research is needed to identify the other possible risk indicators and its relation with the caries experience.

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Master Chart

MASTER CHART

dft ,SUGAR INDEX, SALIVARY FLOW RATE, SALIVARY BUFFERING CAPACITY AND SALIVARY MUTANS STREPTOCOCCUS COUNT IN CARIES GROUP (TEST GROUP)

Sl.No	dft	SUGAR INDEX	SALIVARY FLOWRATE	SALIVARY BUFFERING CAPACITY	SALIVARY MUTANS STREPTOCOCCUS COUNT
1	8	42	0.72	5.5	120
2	4	39	1.20	6.0	50
3	5	35	0.96	5.5	110
4	7	40	0.72	5.5	110
5	4	39	1.20	5.5	50
6	4	35	1.20	5.5	60
7	6	45	0.96	6.0	110
8	5	38	0.96	6.0	110
9	5	37	0.96	5.5	110
10	6	40	0.96	6.0	110
11	7	45	0.72	5.5	110
12	7	45	0.72	5.5	110
13	4	39	1.20	6.0	60
14	4	36	1.20	6.0	50
15	4	38	1.20	6.0	70
16	5	39	0.96	5.5	110
17	4	33	1.20	6.0	50
18	8	48	0.72	5.5	110
19	8	50	0.48	5.0	150
20	4	35	1.20	6.0	110
21	5	32	0.96	5.5	110
22	9	49	0.48	5.0	120
23	8	48	0.72	5.5	110
24	4	30	0.96	5.5	100
25	5	32	0.96	5.5	110

Sl.No	dft	SUGAR INDEX	SALIVARY FLOWRATE	SALIVARY BUFFERING CAPACITY	SALIVARY MUTANS STREPTOCOCCUS COUNT
26	7	42	0.72	5.5	110
27	7	40	0.72	5.5	110
28	3	30	1.20	6.5	30
29	7	41	0.96	5.5	110
30	7	40	0.72	5.5	90
31	3	32	1.20	6.0	20
32	1	31	1.44	7.0	5
33	7	42	0.96	5.5	80
34	3	32	1.20	6.5	30
35	3	30	0.96	5.5	20
36	8	49	0.72	5.5	110
37	2	30	1.20	7.0	7
38	7	41	0.72	5.5	80
39	7	40	0.96	6.0	90
40	2	32	1.20	6.5	5
41	2	31	1.44	7.0	5
42	8	47	0.96	5.5	110
43	8	46	0.48	5.0	150
44	3	30	0.96	5.5	30
45	1	30	1.44	6.5	5
46	3	32	1.20	6.5	30
47	2	31	1.44	6.5	5
48	2	30	1.44	6.5	5
49	3	31	1.20	6.5	20
50	3	32	1.20	6.5	20

MASTER CHART

dft ,SUGAR INDEX, SALIVARY FLOW RATE, SALIVARY BUFFERING CAPACITY AND SALIVARY MUTANS STREPTOCOCCUS COUNT IN CARIES FREE GROUP (CONTROL GROUP)

Sl.No	dft	SUGAR INDEX	SALIVARY FLOWRATE	SALIVARY BUFFERING CAPACITY	SALIVARY MUTANS STREPTOCOCCUS COUNT
1	0	25	1.20	7.0	1
2	0	23	1.68	6.5	1
3	0	27	1.20	7.0	2
4	0	30	1.68	6.5	1
5	0	28	1.92	7.5	0
6	0	29	1.44	7.0	0
7	0	25	1.20	6.5	1
8	0	30	1.20	7.0	0
9	0	26	1.20	7.0	1
10	0	25	1.44	7.0	0
11	0	27	1.20	7.5	3
12	0	28	1.20	7.0	0
13	0	27	1.68	6.5	0
14	0	26	1.20	7.0	2
15	0	26	1.44	7.0	1
16	0	27	1.20	7.0	1
17	0	28	1.44	7.0	0
18	0	27	1.20	7.0	3
19	0	26	1.44	7.5	0
20	0	27	1.68	7.0	1
21	0	27	1.68	7.0	2
22	0	28	1.92	7.0	1
23	0	23	1.92	7.0	1
24	0	26	1.68	7.0	0
25	0	25	1.68	7.5	1

Sl.No	dft	SUGAR INDEX	SALIVARY FLOWRATE	SALIVARY BUFFERING CAPACITY	SALIVARY MUTANS STREPTOCOCCUS COUNT
26	0	25	1.92	7.5	0
27	0	25	1.20	6.5	2
28	0	25	1.20	7.0	1
29	0	26	1.20	6.5	0
30	0	27	1.68	7.0	1
31	0	25	1.92	7.0	1
32	0	25	1.92	7.0	2
33	0	29	1.68	7.0	2
34	0	27	1.92	7.5	1
35	0	27	1.68	7.5	1
36	0	28	1.44	7.0	0
37	0	28	1.44	7.0	1
38	0	26	1.92	7.5	0
39	0	28	1.68	7.0	2
40	0	26	1.68	7.0	2
41	0	23	1.20	7.0	1
42	0	23	1.20	7.0	1
43	0	27	1.92	6.5	2
44	0	29	1.68	7.0	1
45	0	23	1.44	7.0	0
46	0	30	1.20	7.0	2
47	0	22	0.96	6.5	1
48	0	25	1.20	7.0	2
49	0	27	1.20	7.0	0
50	0	29	1.68	7.0	0